ROBERT W. STROZIER, P.L.L.C.
A FIRM SPECIALIZING IN INTELLECTUAL PROPERTY LAW INCLUDING
PATENT, TRADEMARK, COPYRIGHT, TRADE SECRET LAW,
UNFAIR COMPETITION AND RELATED MATTERS

P.O. BOX 429
BELLAIRE, TEXAS 77402-0429
5300 N. BRAESWOOD BLVD. #369
HOUSTON, TEXAS 77096-3317

713.977.7000
713.977.7011/FAX
EMAIL: RWSTROZ@FLASH.NET

FACSIMILE TRANSMITTAL

TO: Examiner CL Smith

OF: USPTO

FAX: 571 273 8300

PHONE: _____

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ROBERT W. STROZIER

USSN: 09/901 782

Docket No.: 00007/01147L

From the desk of...

Sandy

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	**HARDIN ET AL.**	§	ART UNIT NO.: **1633**
SERIAL NO.:	**09/901,782**	§	EXAMINER: **SMITH, CL**
FILED:	**7/9/2001**	§	DOCKET NO.: **00007/01UTL**
		§	
TITLE: **REAL-TIME SEQUENCE DETERMINATION**		§	
		§	

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MS AMENDMENT
571 273 8300

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1 February 2007
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RESPONSE TO 3 NOVEMBER 2006 FINAL OFFICE ACTION

Dear Examiner Smith:

This is a response to the 3 **November 2006** Final Office Action.

SUMMARY OF REJECTIONS

Claim 79	objected - "a site" in singular and later "are not sites"
Claims 10, 13-19 and 79-99	112, first paragraph, - lacking 3' to 5' exonuclease activity
Claims 79 and 89	112, second paragraph - the cysteine
Claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77	102(e) - Korlach et al. + Wisniewski et al.+ Gardner et al.
Claims 79-87 and 89-98	103(a) - Korlach et al. + Wisniewski et al. + Gardner et al. + Schneider et al.

Preliminary Statement

Applicants respectfully disagree with the Examiner's contention that Applicants cannot now antedate the Korlach et al. reference. Applicants made the Examiner aware of Korlach et al. and the Schneider et al. references almost five years ago through an IDS submission. The original Korlach et al. application was filed in the United States and in the PCT in May of 2000, these applications claiming priority to an earlier US provisional filing. The original Schneider et al. application was filed in the PCT designating the United States also claiming priority to an earlier US provisional filing. The Schneider et al. PCT application was later nationalized in the United States.

However, it was only after Applicants received a Notice of Allowance that the Patent Office elected to bring rejections relating to Korlach et al. and Schneider et al. against this application.

Applicants are submitting, via a Rule 131 declaration, antedating documentation as it relates to using beta or gamma labeled dNTPs in sequencing. Applicants believe that this documentation

will be sufficient to antedate the cited Korlach et al. reference as it relates to using beta or gamma labeled dNTPs in sequencing effectively removing the cited Korlach et al. reference. However, even if the Examiner maintains the inclusion of the cited Korlach et al. application in this rejection, Applicants firmly believe that **all of the pending claims are patentable** over the cited Korlach et al. reference alone or in combination with the cited Schneider et al. reference.

The cited Korlach et al. application does disclose beta or gamma labeled dNTPs. However, the Korlach et al. provisional application **DOES NOT** disclose beta or gamma labeled dNTPs. Therefore, as to beta or gamma labeled dNTPs, Korlach et al. is only entitled to the 17 May 2000 filing date and not the provisional filing date of 19 May 1999. Thus, if the Examiner had earlier rejected this application over Korlach et al., Applicants would have been afforded an opportunity to antedate the 17 May 2000 Korlach et al. filing as it related to enzymatic removal of the label from the incorporated nucleotide via its covalent association with the pyrophosphate, and, if needed, Applicants would have been in the midst of an interference or would have had the interference resolved.

Applicants also point out that the beta or gamma labeled dNTP disclosure in the May 17, 2000 Korlach et al. filing does not teach using beta or gamma labeled dNTPs in sequencing reactions involving interactions between a labeled polymerizing agent and the beta or gamma labeled dNTPs such as sequencing reactions based on FRET interactions between beta or gamma labeled dNTPs and a labeled polymerase. Furthermore, the Schneider et al. application is absolutely silent concerning beta or gamma labeled dNTPs. Thus, experts in the art (Korlach et al. and Schneider et al.) did not disclose, teach or even suggest label interactive strategies involving beta or gamma labeled nucleotides and labeled polymerase. This silent more clearly teach away from such a strategy.

A careful reading of both Korlach et al. and Schneider et al., clearly shows that the only FRET strategy either group disclosed involved the use of persistently labeled dNTPs, dNTPs having labels that persist in the resulting DNA strand - labels on the base, sugar or backbone (alpha) phosphate. This fact is clear because both applications teach that the FRET signature will include contributions from all incorporated dNTPs until the extension proceeds to a point where an incorporated and **still** labeled nucleotide is outside of the FRET distance (~60Å in Korlach et al. or 10Å to 100Å in Schneider et al.) or until the labels on the incorporated nucleotides in the resulting DNA strand are photobleached or photocleaved.

On the contrary, when beta or gamma labeled dNTPs are used in a sequencing strategy

involving interactions between a labeled polymerase and labeled dNTPs, there is no need to photobleach or photocleave labels on the resulting DNA strand, because there are no labels on the resulting DNA strand. But neither Korlach et al. nor Schneider et al. disclosed this critical piece of information. Again, Schneider et al. did not even disclose beta or gamma phosphate labeled nucleotides. Again, taken together an ordinary artisan would conclude that these references actually teach away from the such a strategy.

The scientific literature abounds with detection of polymerase activity via labeling the primer strand at the 3' or 5' ends, via 3' labels resulting from incorporation of a base, sugar or alpha-phosphate labeled nucleotides and via 5' labels resulting from primer modification prior to the activity detection step. Less often, the primer strand may be internally labeled via modification of base, sugar or alpha-phosphate. There had been minimal emphasis on non-persistently labeled substrates (labeled on the part of the nucleotide that is released, *i.e.*, the pyrophosphate) and methods using non-persistently labeled nucleotides because there had been no compelling reason to change the emphasis from detection schemes using the above mentioned, persistent labels. Most applications, including traditional DNA sequencing, used base-labeled schemes to detect extension products.

Further, most researchers at the time of Applicants filing believed that nucleotides modified at the beta or gamma phosphate would not produce extension products – rather they would act as terminators of enzyme activity. The following text from Kao et al. supports this belief and its logic was previously accepted by the USPTO, resulting in issuance of US patent 6,399,335:

> Several companies sell products that incorporate a detectable reagent into the product of polymerase synthesis, including Boehringer (Genius kit), Life Technologies INC., GIBC/BRL, Sigma (biotinylated nucleotides, fluorescent nucleotides), Molecular Probes Inc. (a large range of fluorescent and caged nucleotides), Li-Cor (dyes attached to DNAs for DNA sequencing), etc. Reports of γ-phosphoesters of nucleoside triphosphates have described them as non-hydrolyzable and used them in solid phase affinity purification protocols, e.g. Clare M. M. Haystead, et al., Gamma-phosphate-linked ATP-Sepharosefor the affinity purification of protein kinases, Eur. J. Biochem. 214, 459-467 (1993), esp. p.460, col. 2, line 23. We synthesized large numbers of γ-phosphoester nucleoside triphosphates and found that while they are indeed non-hydrolyzable by many enzymes, they are often suitable substrates for RNA and DNA polymerases.

Applicants note that Kao et al. provided an example of a research report that describes gamma labeled nucleotides as non-hydrolyzable for certain enzymes. Thus, ordinary artisans would not consider such labeled nucleotides as good monomers for making a DNA strand, and clearly not an

obvious choice for labeling if you want the polymerase to continue DNA synthesis.

Thus, at the time of Applicants' filing, ordinary artisans would not view beta or gamma labeled nucleotides as substrates in single molecule sequencing. This fact is reinforced by the cited Korlach et al. and Schneider et al. references. Neither reference nor a combination thereof disclosed a sequencing strategy using beta or gamma labeled dNTPs and a labeled polymerase in a label interactive context such as a FRET, *i.e.*, the specifications of Korlach et al. and/or Schneider et al. **DO NOT SUPPORT** sequencing strategies involving interactions between non-persistent labeled dNTPs and a labeled polymerase. However, Korlach does include mention of beta or gamma labeled nucleotides in their May 2000 application.

Applicants' sequencing technology based on detecting transient interactions between beta and/or gamma labeled dNTPs and labeled polymerizing agents is conceptually and fundamentally different than the Korlach et al. and Schneider et al. sequencing technologies based on detecting much longer lived interactions between base, sugar or alpha phosphate labeled dNTPs (persistently labeled dNTPs) and labeled polymerases. Moreover, Applicants' sequencing technology using non-persistently labeled dNTPs generates nascent natural (non-modified) DNA duplexes, while the Korlach et al. and Schneider et al. sequencing technologies using persistently labeled dNTPs generate labeled DNA duplexes. These labeled DNA duplexes are generally treated post incorporation to permit continued extension – cleaving or bleaching of the labels – as was recognized by both groups for the successful implementation of these technologies; otherwise, the labels on the DNA duplexes continue to contribute to the detected signal complicating data acquisition and analysis. Additionally, the modification on the nucleotide analog – the labeled, incorporated nucleotide – tends to reduce the efficiency of subsequent incorporation events.

In fact, because both groups failed to disclose Applicants' sequencing technology, the cited references, taken together, teach firmly away from Applicants' sequencing technology.

Applicants also do not believe that the Examiner is justified in citing claims in the cited Korlach et al. application against Applicants' present claims. The claims of the cited Korlach et al. application find absolutely no support in the Korlach et al. specification. Applicants, respectfully, point out that only the original claims of the parent Korlach et al. application form a part of the original specification. The claims of the cited Korlach et al. application were essentially copied from Applicants' application. The Examiner may only rely on this new Korlach et al. claim set as prior art, if they find support in the original Korlach et al. specification, which they do not. The only

tag interactive strategy disclosed by Korlach et al. and Schneider et al. for that matter is a strategy based on persistently labeled nucleotides and not non-persistently labeled nucleotides.

Moreover, Applicants' antedating document removes Korlach et al.'s ability to claim use of beta or gamma labeled nucleotides in any sequencing strategy.